

Osteological structure of Kiabi loach, *Oxynoemacheilus kiabii* (Actinopterygii: Nemacheilidae)

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Abstract: A detailed description of the osteological structure of Kiabi loach (*Oxynoemacheilus kiabii*), a recently described endemic species caught from Gamasiab River, Iran, is provided. Seven specimens were collected by electrofishing in June 2013 and cleared and stained and its osteological structures were described. The results showed that *O. kiabii* can be distinguished from other members of the genus *Oxynoemacheilus* by having a square prevomer versus elongated and rectangular prevomer of *O. bergianus*, wider and shorter supraethmoid-ethmoid, shorter connection area between prootics, presence of two sesamoids bones, larger processes of hypohyals, presence of two extra urohyals, short metapterygoid, shape of swim bladder capsule and five hypurals.

Keywords: Skeleton, Loach, Neurocranium, Nemacheilidae.

Introduction

The members of nemacheilid family are distributed from Eurasia to northeast Africa (Ethiopia) (Prokofiev 2009; Coad 2014). Forty-six percent of Iranian loach species is considered as endemic species (Abdoli et al. 2011), indicating their importance. The genus *Oxynoemacheilus* is a species-rich taxa of nemacheilid fishes found from Albania eastwards to central Iran (Freyhof et al. 2011), which has 13 reported and only eight confirmed species in Iran (Coad 2014). Hence, this genus needs to be fully explored and more species expected to be recognized (Kamangar et al. 2014; Freyhof et al. 2011).

Kiabi loach, *Oxynoemacheilus kiabii*, Golzarianpoor, Abdoli & Freyhof, 2011, was introduced only recently. The genus *Oxynoemacheilus* is distinguishing by having two lateral pores on each side of the supratemporal canal without central pores, but with no suborbital flap in males, a slightly emarginated caudal fin and an incomplete body lateral line system (Golzarianpoor

et al. 2011). A comprehensive osteological study of nemacheilid family is performed by Prokofiev (2010) providing a basic osteological characteristics of this family. Hence, the recent classification of nemacheilid family was established based on osteological features (Prokofiev 2009, 2010). Since, there is no information available on the osteology of *O. kiabii*, therefore, this study was conducted to provide a detailed description of its osteological features. The osteological along with molecular features are important characteristics in taxonomy, particularly in nemacheilid family, giving better understanding of their taxonomical circumstances. Therefore, the results of this study can be used as a reference for comparison with other *Oxynoemacheilus* species.

Materials and methods

Seven specimens of *O. kiabii* (Fig. 1) with a standard lengths of 36.78, 40.78, 40.42, 37.12, 34.31, 36.4 and 40.32mm were collected from Gamasiab River (Kermanshah Province, Iran) on 21 June 2013. The



Fig.1. Lateral view of *Oxynoemacheilus kiabii*.

collected fishes were fixed in 4% buffered formalin after anesthetizing in 1% clove oil solution. Then, specimens were cleared and stained with alizarin red S and alcian blue according to the protocol of Taylor and Van Dyke (1985). The cleaned and stained specimens were studied using a stereomicroscope (Leica MC5) and different skeletal elements were dissected and scanned by a scanner equipped with a glycerol bath (Epson v700). The obtained images were drawn using CorelDrawX6 software. Terminology of skeletal elements follows Prokofiev (2009, 2010). The cranial osteology of *O. bergianus* and *O. angorae* was described by Jalili and Eagderi (2014) and Prokofiev (2010), respectively and their information were used for comparison.

Results

Neurocranium: The anterior part of neurocranium is narrow with flat posterior part. The maximum width of skull is at the level of pterotics. The length of orbito-rostral region is almost equal to that of postorbital one. The maximum length of the neurocranium is 1.6 folds of its maximum width. The ethmoid region consists of the two unpaired prevomer and supraethmoid-ethmoid and paired lateral ethmoid bones (Figs. 2a, 2b). The supraethmoid-ethmoid is short and wide and its width is one-third of its length. This bone is vertically fused to the prevomer and posteriorly connected to the frontal (Figs. 2a, 2b). The prevomer is almost square-shaped bearing two anterolateral processes connected to the preethmoid-II anteriorly (Fig. 3), and orbitosphenoid posteriorly (Fig. 2c). The lateral ethmoid possesses a concave-shape anterior process and a convex-shape posterior one. The lateral

ethmoid is not completely ossified, and joined laterally to the orbitosphenoid (Fig. 2b). A ligamentous connection present between the lateral-ethmoid, prevomer and parasphenoid. In addition, the lateral ethmoid is connected to frontal forming the superior rim of orbital (Fig. 3).

There is small bone series at the anterior position of ethmoid region. They consist of paired preethmoids-II, prepalatines and sesamoids and unpaired kinethmoid (Fig. 3). The preethmoid-II is small and rod-like, locating between the prevomer and maxilla (Fig. 3). The prepalatine positions between the autopalatine and maxilla (Fig. 3). The preethmoid-II is connected to the prepalatine laterally. Also, two small and circle sesamoid bones are observed in the ethmoid region (Fig. 3). The kinethmoid is a small cylindrical bone, with narrow middle part.

The orbital region is composed of the frontal, orbitosphenoid, pterosphenoid, parasphenoid and sclerotic (Fig. 2). Also, the otic region consists of the pterotic, epiotic, prootic, sphenotic and parietal (Fig. 2). The largest bone of skull roof is the frontals. In the midline, these bones are connected irregularly and in their lateral rim is narrow and non-tubular, forming the roof of orbit. The frontals are connected to the orbitosphenoid, pterosphenoid and sphenotic laterally, and parietal posteriorly (Figs. 2a, 2b). The parietal is connected to the sphenotic anterolaterally, the epiotic posterolaterally and the supraoccipital posteriorly (Fig. 2a). The relationship between the parietal and pterotic is absent due to connection of the sphenotic and epiotic which is an apomorphic state in loaches. The pterotic is triangular which its ventral edge is curved. The frontal, parietal and supraoccipital enclose the fontanelle of neurocranium (Fig. 2a). The orbitosphenoid is almost concaved connecting to the heavy bone of pterosphenoid (Fig. 2b). The orbitosphenoid and pterosphenoid form a pore ventroposterior in which its ventroposterior is projected (Fig. 2b).

In the ventral face of neurocranium, the parasphenoid is largest element with bifurcated ends.

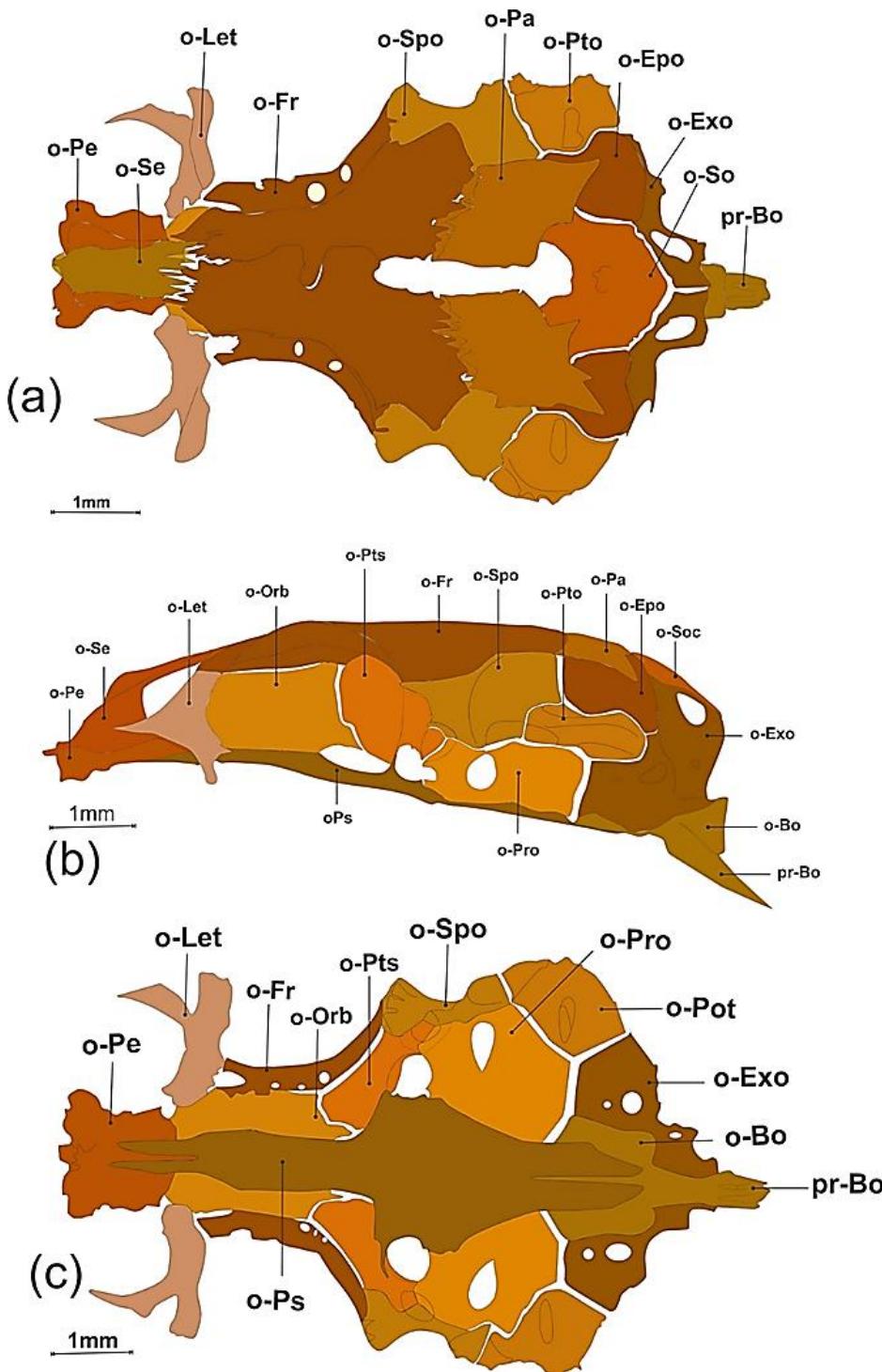


Fig.2. Neurocranium of *Oxynoemacheilus kiabii*. (a) Dorsal view, (b) lateral view and (c) ventral view. pr-Bo: basioccipital process; Bo: basioccipital; Epo: epiotic; Exo: exoccipital; Fon: fontanelle; Fr: frontal; Fr-Exo: foramen exoccipital; Let: lateral ethmoid; Orb: orbitosphenoid; Pa: parietal; Pe: prevomer; Pro: prootic; Ps: parasphenoid; Pto: pterotic; Pts: pterosphenoid; Se: supraethmoid-ethmoid; Soc: supraoccipital; Spo: sphenotic.

The parasphenoid is laterally connected to the pterosphenoid and prootic forming its anterior foramen and posterior to anterior foramen, there is, also a prootic pore (Fig. 2c). The prootic is a large

and flat bone which connected to the pterosphenoid and sphenotic anterolaterally (Fig. 2c). The sphenotic, pterosphenoid and prootic contribute in formation of the first facet of hyomandibular condyle

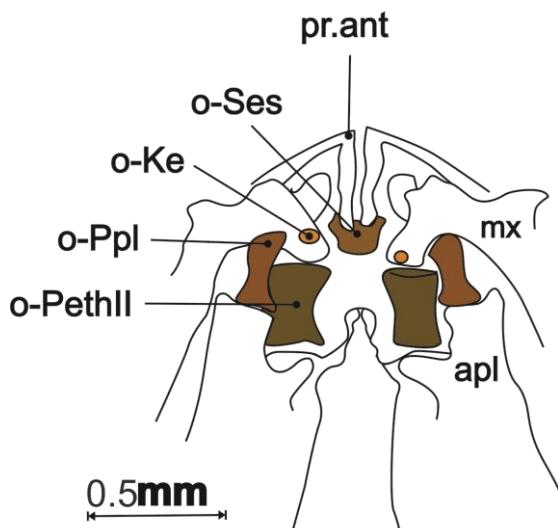


Fig.3. Dorsal view of ethmoid region of *Oxynoemacheilus kiabii*. Ke: kinethmoid; Peth II: preethmoid II; Ppl: prepalatine; pr.ant: anterior processes of the premaxilla; Ses: sesamoid.

(Figs. 2b, 2c). Also, the second facet of hyomandibular condyle is formed by the sphenotic and pterotic (Figs. 2b, 2c).

The occipital region consists of the supraoccipital, exoccipital and basioccipital (Figs. 2a, 2c). The supraoccipital bears an elongated lateral process superimposed by the parietal and connected to the sphenotics. The paired exoccipitals form the posterior face of skull, connecting to the prootic ventroanteriorly (Fig. 2c). The exoccipital bears foramen exoccipitalis. The basioccipital has a ring-like process posteriorly (process basioccipital) (Fig. 2c). This bone also is positioned between the two exoccipitals and connected to the prootic anteriorly (Fig. 2c).

Jaws: The upper jaw consists of the premaxilla and maxilla (Fig. 4a). The premaxilla is an L-shape bone with two processes, i.e., ascended and alveolar premaxilla processes. The vertical process of premaxilla is narrower than horizontal one. The horizontal process is bow-shaped, whereas the vertical one is almost straight. The width of maxilla at two ends is greater than its middle part. There is a triangular process in inferomedial margin of maxilla. The lower jaw is composed of the dental, articular, retroarticular and coronomeckelian (Figs. 4b and 4c).

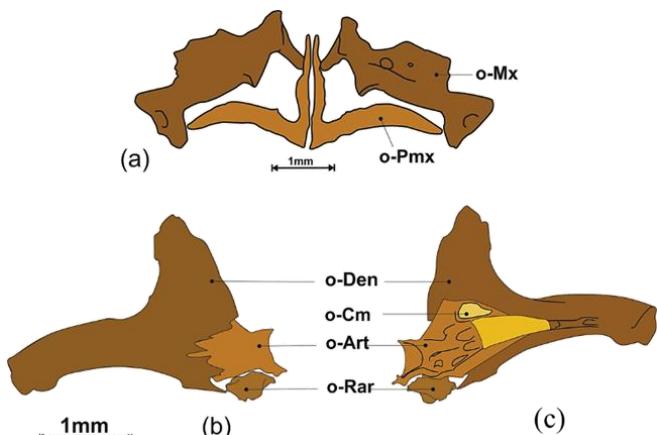


Fig.4. (a) Upper jaw, (b) lateral view of lower jaw and (c) medial view of lower jaw in *Oxynoemacheilus kiabii*. Art: articular; Cm: coronomeckelian; Den: dental; Mx: Maxilla; Pmx: Premaxilla; Rar: retroarticular.

The dental can be divided into two parts including narrow ramus dentalis and wider coronoid process. The anterior part of articular is wider than its posterior part that is articulated to the quadrate. The articular is ventrally connected to a small retroarticular bone. There is a small coronomeckelian which is located on the dorsomedial of articular.

Branchial arches: The branchial arch consists of the basibranchial, hypobranchial, ceratobranchial, epibranchial, and infrapharyngobranchials (Fig. 5). There are four unpaired basibranchials. The elongated ceratobranchials are the largest elements of branchial arch, that fifth of them modified as pharyngeal teeth. Three paired hypobranchials are positioned between the ceratobranchial and basibranchial bones. There are four paired of epibranchials which are connected to ceratobranchials posteriorly and infrapharyngobranchials anteriorly. The anterior part of epibranchial is narrower than theirs posterior part. The infrapharyngobranchials are directed horizontally. There are three pairs of infrapharyngobranchials in which second one is the largest one (Fig. 5).

Hyoid arch: The hyoid arch is composed of the basihyal, dorsal hypohyal, ventral hypohyal, ceratohyal, epihyal, interhyal, urohyal, extra urohyal

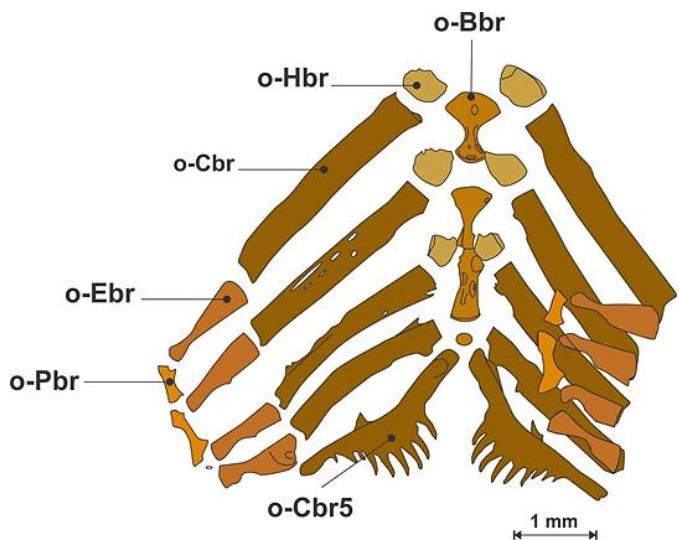


Fig.5. Branchial apparatus of *Oxynoemacheilus kiabii*. Bbr: basibranchial; Cbr: ceratobranchial (cbr5: pharyngeal bones); Ebr: epibranchial; Hbr: hypobranchial; Pbr: infrapharyngobranchials.

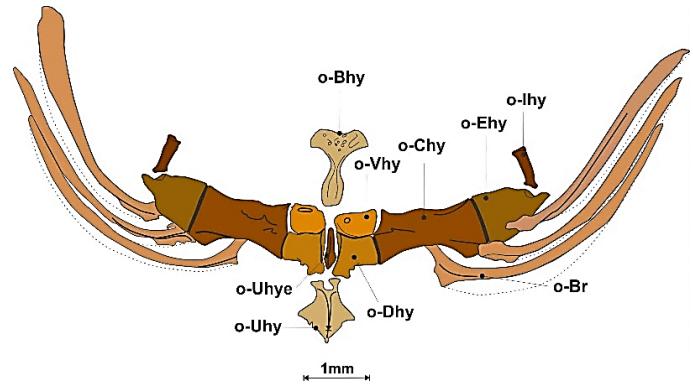


Fig.6. Hyoid arch of *Oxynoemacheilus kiabii*. Bhy: basihyal; Br: branchiostegale; Chy: ceratohyal; Dhy and Vhy: dorsal and ventral hypohyal; Ehy: epihyal; Ihyl: interhyal; Uhy: urohyal; Uhye: urohyal extra.

and branchiostegals (Fig. 6). The unpaired basihyal is T-shape and its anterior margin is slightly round. The ventral and dorsal hypohyal are tightly connected and both have a processes which process of ventral hypohyal is larger than that of dorsal one. The ceratohyals and dorsal hypohyal forms a distinct groove on their dorsal faces. The epihyal is subtriangular having a depression on its dorsal end that connected to the small, rod-shape interhyal bone. The unpaired urohyal is laminar in dorsal face and possesses two wing-shape processes ventrolaterally and a hole anteroventrally. There are two extra urohyals between hyporals and below urohyal. The branchiostegals are three in number and first one is wider than others. The first branchiostegalis is connected to the ceratohyal dorsally, second one to the ceratohyal and epihyal ventrally and third one to epihyal ventrally (Fig. 6).

Suspensorium: The suspensorium is composed of the autopalatine, endopterygoid, ectopterygoid, metapterygoid, hyomandibular, quadrate and symplectic (Fig. 7). The autopalatine is a heavy bone bearing the wings in its middle part laterally that connected to the prevomer. Furthermore, this bone bears two processes, which are connected to the

prepalatine and preethmoid anteriorly and to the endopterygoid posteriorly. The endopterygoid is a wide and elongated bone connected to the ectopterygoid and metapterygoid via its ventral margin. The metapterygoid is a flat bone and its anterior is connected to the quadrate. The metapterygoid, hyomandibular, symplectic and interhyal are connected by a ligamentous sheet covering their medial faces. The dorsal margin of hyomandibular is wider than its ventral part. The hyomandibular possesses two condyles on its dorsal edge (anterior and posterior hyomandibular condyles) which are articulated to the neurocranium. Also, the opercular condyle presents at the posterodorsal margin of hyomandibular. The anterior part of quadrate is larger than posterior part. The quadrate condyle is situated on the anterior edge of this bone. The quadrate is connected to the small bone of ectopterygoid anteriorly. The triangular bone of symplectic is placed into the incisures of quadrate.

Opercular series: this series consist of four elements including opercle, subopercle, interopercle and preopercle (Fig. 7). The opercle is the largest element of opercle series and has a triangle-shape. The preopercle is elongated and curved and covers the posterior rims of hyomandibular and quadrate. The interopercle is a long bone located dorsal to preopercle.

Pectoral girdle: The pectoral girdle consists of the

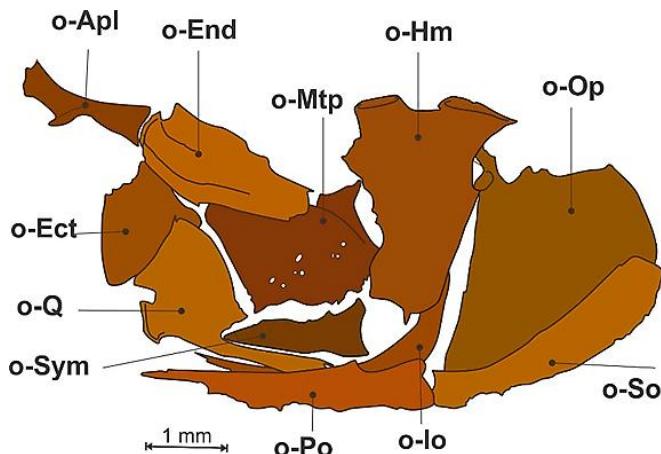


Fig.7. Lateral view of suspensorium in *Oxynoemacheilus kiabii*. Apl: autopalatine; Ect: ectopterygoid; End: endopterygoid; Hm: hyomandibular; Io: interopercle; Mtp: metapterygoid; Op: opercle; Po: preopercle; Q: quadrate; So: subopercle; Sym: symplectic.

coracoid, mesocoracoid, scapula, cleithrum, supracleithrum, posttemporal, supratemporal and radials (Fig. 8). The small bone of supratemporal is fused to the elongated posttemporal (Fig. 8c). The supratemporal and posttemporal are connected to the supracleithrum. The supracleithrum is wide and its anterior part has a dorsally projected process. The anterior part of supracleithrum has an elongated process. This bone is connected to the cleithrum. The scapula possesses a large opening (scapula foramen) and is connected to the cleithrum and coracoid. The largest element of pectoral girdle is the cleithrum that has a laminar shape. The cleithrum is anteroventrally connected to the coracoid. A small mesocoracoid is connected to the coracoid and cleithrum medially. The radials are four in number and the longest one is first one and shortest is the fourth. The first three radial have similar width and last one is the widest one. The pectoral fin has 11-12 rays (Fig. 8b). The examined specimens had 11-12 rays, whereas Golzarianpour et al. (2011) is reported its number as 10.

The pelvic girdle: The pelvic girdle is composed of the paired pelvic bones and radials (Fig. 9c). The radials are small and round bones which are positioned between the pelvic bones and rays and

three in number (in one of specimen four radials were observed). The anterior part of pelvic bone is narrower than the posterior part. The posterior face of pelvic bones is thick with many tiny depressions. The pelvic bones have three processes including the anterior process that has some depression laterally (pubic process), the posterolateral process that rays connected on it (iliacus process) and the posterioral process (ischiadicus process). There is one unbranched ray and 7 branched rays with one free small ray (pelvic splint).

Unpaired fins: There are 9 pterygiophores in the dorsal fin (Fig. 8a). The dorsal fin has 4-5 unbranched and 8½ branched rays (Fig. 8a). The first pterygiophor is the largest one and often adjacent to 11 and 12 centra. The anal fin is composed of 6-7 pterygiophores, 1 stay, 4-5 unbranched rays and 5½-6½ branched rays (Fig. 9b). The first pterygiophores is usually adjacent to 23th and 24th centra.

Vertebral column: The centra are cylindrical in shape and the four anterior centra form Weberian apparatus and bony capsule. The vertebral is 34-36. The bifurcated neural and hemal of processes are observed among the vertebra such as hemal process of 33th centrum and neural process of 31th centrum.

Caudal skeleton: The first preural and first and second ural are fused forming the last centrum. There are five hypurals that the first one along with the parhypupophysis is autogenous (Fig. 10). The parhypupophysis is present. The hypural 2 and pleurostyl are tightly connected to the centrum. The shape of epural was variable between examined specimens having several states. In some specimens, this bone was absent. When the epural was present, it could be connected to the pleurostyl or not. Also, the epural was free in some examined specimens. The neural and haemal processes of preural 2 are larger than that of preural-1.

Weberian apparatus and swim bladder capsule: The weberian apparatus is composed of four weberian ossicles and four vertebral elements (Fig. 11). The weberian ossicles include the claustrum, scaphium, intercalarium and tripus (Fig. 11). The claustrum is

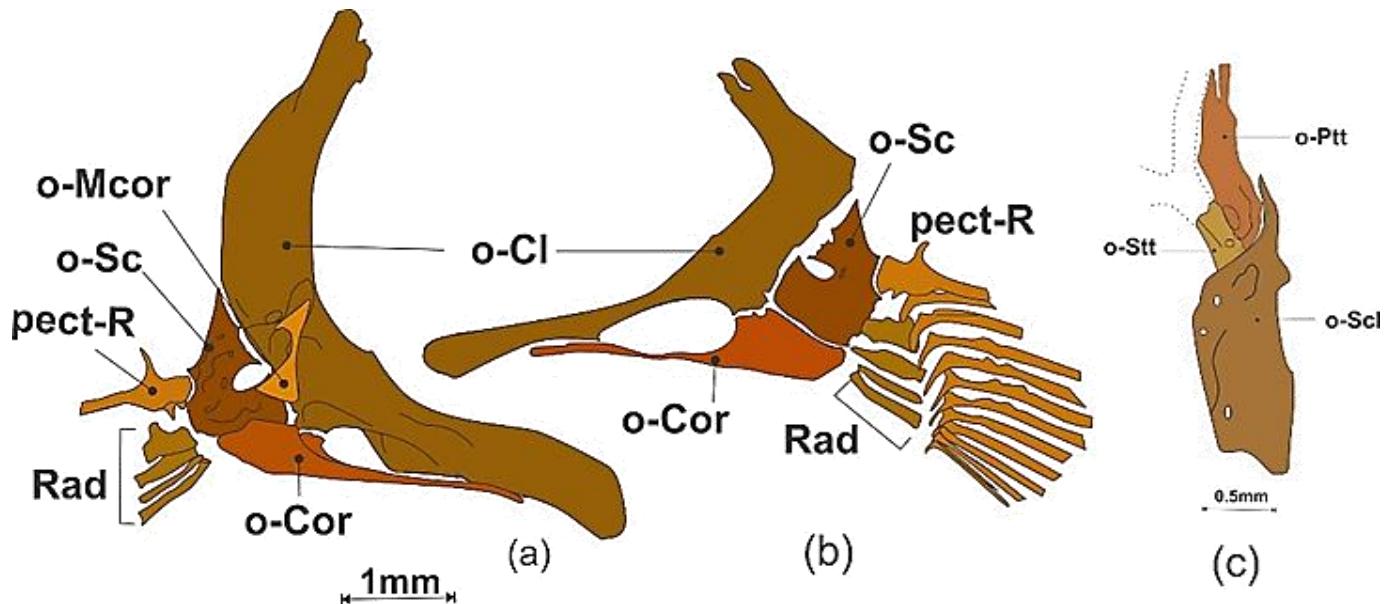


Fig.8. Pectoral girdle of *Oxynoemacheilus kiabii*. (a) Medial view, (b) lateral view of pectoral girdle and (c) lateral view of supracleithrum and supratemporal. Cl: cleithrum; Cor: coracoid; Mcor: mesocoracoid; pect-R: ray of the pectoral fin; Ptt: posttemporal; Rad: ossified pectoral radial; Sc: scapula; Scl: supracleithrum; Stt: supratemporal.

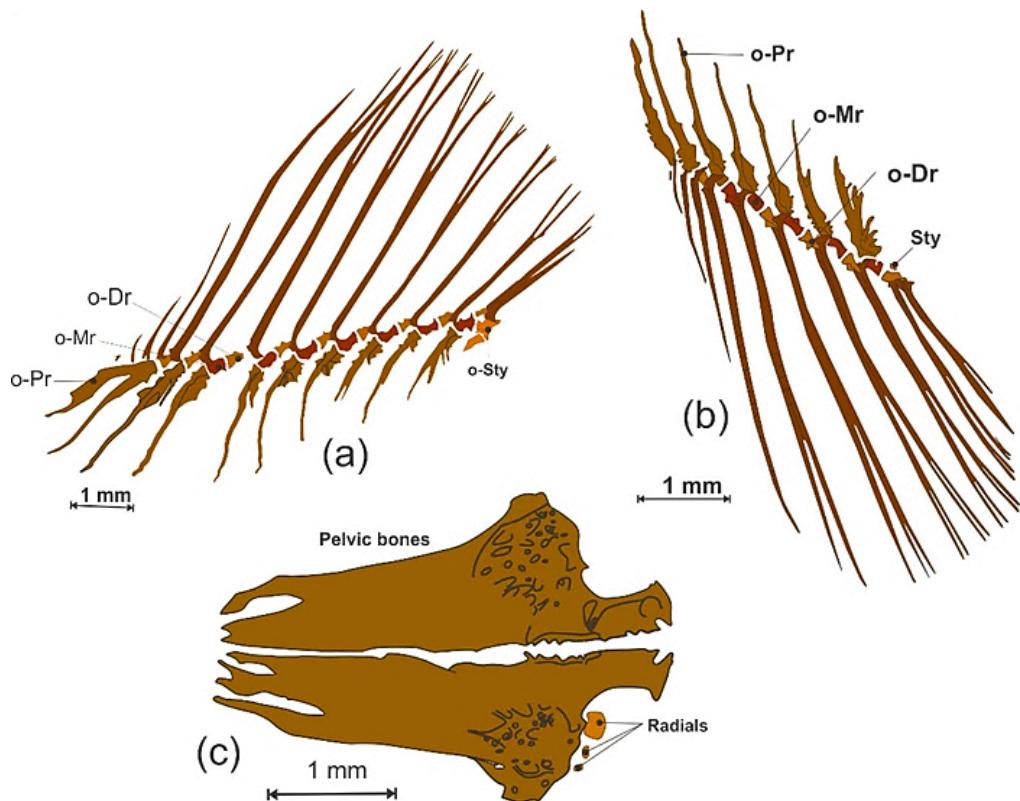


Fig.9. (a) Dorsal fin, (b) anal fin and (c) pelvic girdle of *Oxynoemacheilus kiabii*. Dr: distal radial; Mr: medial radial; PR: proximal radial; Sty: stay.

elongated and covers bowl-shape scaphium ventrally. Also the claustrum is dorsally connected to the supraneural-2. The intercalarium is small and

positioned between the scaphium and Y-shape tripus. The first centrum of the trunk does not participate in the formation of the bony capsule which has two

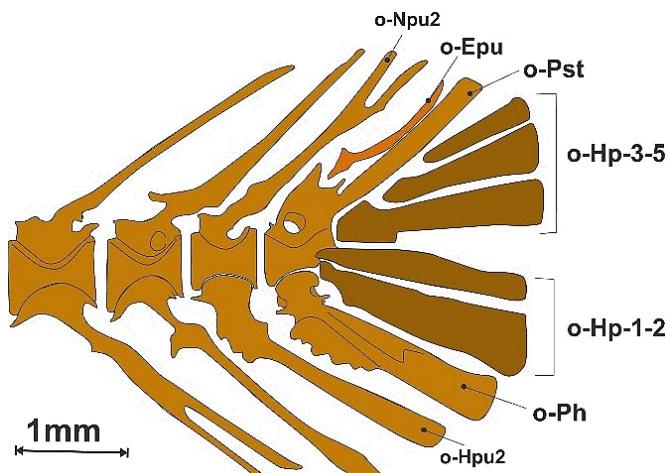


Fig.10. The caudal skeleton of *Oxynemacheilus kiabii*. Epu: epural; Hp: hypural; Hpu2: hemal process of the second preural centrum; Npu-2: neural process of the second preural centrum; Ph: parhypural; Pst: pleurostyle.

lateral processes connected to the pectoral girdles by ligaments. The second, third, and fourth centra participate in the formation of bony capsule. The second and third centra are fused. The second lateral process of centrum-2 forms second horizontal process and second descending process which form anterior part of swim bladder capsule. The third centrum is connected to the supraneural-2 anteriorly and to the fourth centrum posteriorly. The parapophysis of forth centrum is modified and has similar processes as second centra. In lateral view, an anterior and a posterior openings are observed that posterior one is larger (Fig. 11). In ventral view, the bony capsule is divided into two lobe connected by manubrium.

Discussion

Oxynemacheilus kiabii shows some differences with two other member of the genus *Oxynemacheilus*, i.e., *O. angorae* and *O. bergianus* in the neurocranium, suspensorium, hyoid arch, swim bladder capsule and caudal fin skeleton. According to Prokofiev (2010), the lateral ethmoid of loaches is stationarily jointed with the supraethmoid-ethmoid, whereas such a state is not observed in *O. kiabii* and *O. bergianus*.

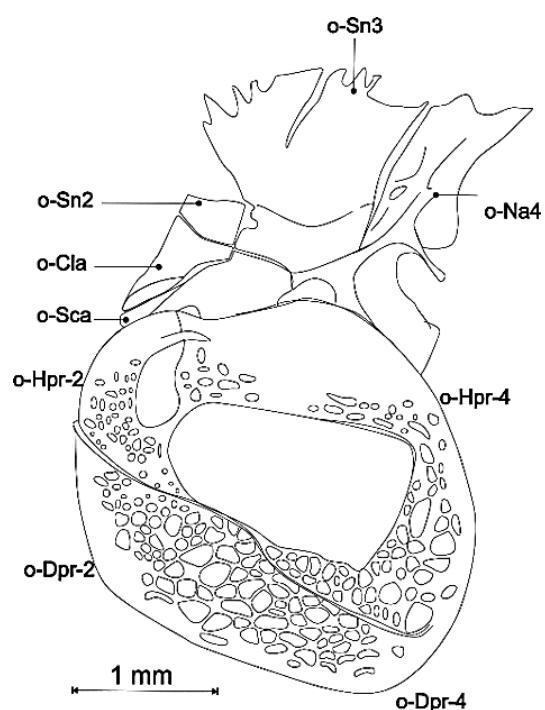


Fig.11. The swim bladder capsule of *Oxynemacheilus kiabii*. Cla: claustrum; Dpr-2 -4: descending processes of the second and fourth centra; Hpr-2-4: horizontal processes of the second and fourth centra; Na4: neural arch 4; Sca: scaphium; Sn2: supraneural 2; Sn3: supraneural 3.

The prevomer of *O. kiabii* is square-shape, while this bone is elongated and rectangular in *O. bergianus*. Also, the supraethmoid-ethmoid of *O. kiabii* is wider and shorter than that of *O. bergianus*. The key feature for distinction of *O. kiabii* from others member of the genus *Oxynemacheilus* is absence of the central pore in supratemporal canal (Golzarianpour et al. 2011). Also, the prootics have shorter connection area in *O. kiabii* than *O. bergianus*.

The anterior and posterior facet of hyomandibular are formed by the sphenotic and prootic; and the sphenotic, prootic; and pterotic in *O. angorae*, respectively (Prokofiev 2010), while these two facets are formed by the prootic, pterosphenoid and sphenotic; and pterotic and sphenotic in *O. kiabii* and *O. bergianus*, respectively. In addition, there are two sesamoids bones in *O. kiabii*, which are previously rejected by Sawada (1982) (Prokofiev 2010).

Prokofiev (2010) pointed out that the coronomeckelian is connected to the base and dorsal edge of the coronoid process in the members of the genus *Oxynoemacheilus*, whereas this bone is connected to the medial face of articular. Also, in *O. kiabii*, the metapterygoid is short and square-shape, while this bone is elongated and rectangular in *O. bergianus*.

In *O. kiabii*, the processes of hypohyals (dorsal and ventral) are larger than that of *O. bergianus*. There are two extra urohyals in *O. kiabii* and *O. bergianus*, which are not already reported by Prokofiev (2010) for the member of this genus.

The most important differences of *O. kiabii* from *O. angorae* and *O. bergianus* is the number of hypurals and the shape of bony capsule. *Oxynoemacheilus kiabii* has five hypurals, however, the two others have six hypurals. In *O. kiabii*, bony capsule is round with high fenestrated surface but that of *O. bergianus* is rectangular. Also, the posterolateral opening of this capsule is larger in *O. kiabii* than that of *O. bergianus*.

As a conclusion, *O. kiabii* is distinguished from other members of the genus *Oxynoemacheilus* by having square-shape prevomer, wider and shorter supraethmoid-ethmoid, the rounded shape of the swim bladder capsule, shorter connection area between prootics, presence of two sesamoids bones, larger processes of hypohyals, presence of two extra urohyals, short metapterygoid and five hypurals.

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